Association between sperm MT-COX1 gene variations and semen quality in the Chinese Han population

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Research Article

Keywords: Chinese, Mitochondrial DNA, Variation, Sperm density, Sperm motility

Posted Date: May 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1683020/v1

Abstract

Backgroud: Male sperm quality has declined as a whole in the last 30 years and our previous study found that there are many specific variations in male sperm mitochondria, including MT-COX1. In this study we attempted to explore the correlation between human sperm MT-COX1 gene variations and male semen quality in a Chinese Han population.

Methods and results: There were 477 male patients from the Second Affiliated Hospital of Zhengzhou University from 2010 to 2021, of whom 331 were retrospective studies and 146 were prospective studies. And 4 oligoasthenoteratospermia samples and 13 normal sperm samples were collected for low-throughput sequencing of the whole human genome. The semen samples were divided into three groups according to the types of genetic variation at the m.6023G>A locus: G/G genotype (wild type), G/A genotype and A/A genotype. The results of a prospective study show that the A/A genotype had higher sperm density and motility than the G allele carrier (G/G+G/A). And DNAAF6 gene variation may be associated with oligoasthenoteratospermia, the upstream of the 107243978 position of DNAAF6 gene variation might have the binding of hsa-miR-4311 to the 3'UTR of DNAAF6 gene.

Conclusions: We first found that the MT-COX1 gene of spermatozoa of the Chinese Han population has varied since 2014. Furthermore, the A/A genotype had better sperm quality than the G allele carriers (G/G+G/A), and the 107243978 position variation of the DNAAF6 gene might affect the gene expression by affecting the stability of transcripts.

1. Introduction

Infertility is a state of reproductive disorders caused by both male and female factors, and it is an adverse event harmful to human reproductive health. In recent years, in addition to infertility caused by female factors, infertility caused by male factors has become increasingly common [1]. There was no significant difference in the incidence of infertility among different races and regions. According to a survey conducted by the World Health Organization, the proportion of infertility in the world accounted for 10% of the population of childbearing age, of which the male factor alone contributed up to 40% of cases of infertile couples [2]. A meta-analysis showed that the male sperm density, motility and fertility declined by more than 50% in developed countries [3].

There are many reasons for the decline in sperm quality, including exposure to toxic environments, varicoceles, physical factors and mental factors. With the advancement of molecular biology and genetic research, some researchers have further turned their attention to sperm mitochondria in recent years. Several studies found a close relationship between mitochondrial gene fragment deletion and male infertility [4–10]. In our previous studies, we found that variants of the mitochondrial genes MT-ATP6, MT-ND3 and MT-ND6 correlated with complete fertilization failure in vitro [11–13]. However, we still knew little about the correlation between sperm MT-COX1 gene variation and male sperm quality, which we attempt to explore in this study.

Mitochondria are the energy factories of cells and play an important role in the energy production and regulation of substance metabolism. There is a mitochondrial sheath in the tail of the sperm, which contains 22–72 mitochondria. Sperm mitochondria play an indispensable role in spermatogenesis, movement and fertilization. Mitochondrial DNA is a 16569 bp double-stranded closed circular molecule encoding 13 proteins, 22 tRNAs and 2 rRNAs. Thirteen protein-coding regions can be further divided into ND1-ND6-encoding complex I, Cytb-encoding complex III, COX1-COX3-encoding complex IV, ATP6 and ATP8-encoding ATP synthase. The COX gene encoding complex IV is an important rate-limiting enzyme in the oxidative respiratory chain. Therefore, the variation, deletion or copy number change of the sperm MT-COX gene might seriously affect the function of the respiratory chain, cause ATP production disorder, affect the sperm quality and reduce the male fertility.

2. Materials And Methods

2.1. Sample Collection

There were 477 male patients from the Second Affiliated Hospital of Zhengzhou University from 2010 to 2021, of whom 331 cases were retrospective studies and 146 cases were prospective studies. Six male volunteers and their biological mothers in 2018 were recruited, and semen samples and blood samples were taken from each individual and sequenced. The semen samples were divided into three groups according to the types of genetic variation at the m.6023G > A locus: G/G genotype (wild type), G/A genotype and A/A genotype. The effects of the MT-COX1 gene variation on the sperm quality were analyzed and discussed.

2.2. Mt-DNA Extraction

Semen samples were obtained by masturbation after 3–7 days of abstinence. The samples were liquefied in an incubator at 37℃. The liquefied spermatozoa were centrifuged with a double-layer density gradient centrifugation solution (40% and 80% Purception), and the spermatozoa with different motility were separated in the gradient centrifugation solution. Then, approximately 7×10^6 sperm were washed with 5% 1026 and centrifuged at a speed of 200 × g for 8 min. The supernatant was discarded, and the precipitate used for DNA extraction was preserved at -80°C. In the DNA extraction process, the precipitate was digested with a total volume of 600 μL cleavage buffer containing 1% SDS and 0.2 mg/mL K protease. The mixture was cultured for 40 min at 60°C, 800 μL pure ethanol was added, the diluent was mixed, and the mixture was centrifuged for 1 min at 11100 × g. The supernatant was discarded, the precipitate was diluted with 700 μL cleaning solution and subsequently centrifuged for 1 min at 11100 × g. Finally, the separated DNA precipitate was dried in air and added to 50 μL ddH_2O.

2.3. Nested PCR and Sequence Analysis

The corresponding primers were designed and synthesized according to the gene sequence of interest. Primer sequences and nested PCR conditions and their GenBank Accession sequences (Table 1). All sample genes were analyzed by Surveyor V5.0.2 software. The human Cambridge sequence (rCRS)

provided in the Mitomap (www.mitomap.org) database and dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) database were used to compare and verify the test results and find the variations. Whether the site caused a change in the encoded amino acid was recorded and analyzed.

Table 1
PCR and nested PCR primers, reaction conditions and Genbank accession numbers

Gene	Primers	Product (bp)	Annealing temperature (°C)	n of cycles	Accession numbers
MT-COX1 Outer	F:CTGAACGCAAATCAGCCACT	2220	56	30	NC-012920
	R:AAAGGATGCGTAGGGATGG				
Inner	F:AGCACCCTAATCAACTGGC	1901	55	35	NC-012920
	R:TGCGCTGCATGTGCCATTA				

2.4. Statistical Analysis

Statistical analysis was performed using the SPSS (Statistical Package for the Social Science), version 25.0 software for Windows. Continuous count data are expressed as the mean ± standard deviation, and the number and percentage of classified count data are expressed. Normally distributed variables were analyzed using the independent samples t-test, nonnormally distributed variables were analyzed using Mann–Whitney test, and dichotomous variables were studied using Fisher's exact test or chi-square test. All analyses were performed on both sides, and $P < 0.05$ was considered statistically significant.

2.5. Ethics

Informed consent

was obtained from all participants and this study was approved by the Research Ethics Committee of Zhengzhou University.

3. Results

3.1. MT-COX1 Gene Variation and Functional Impairment of Variant Gene Proteins

We collected 477 sperm samples from 2010 to 2021, of which 331 cases were retrospective studies and 146 cases were prospective studies. In total, 75 homozygous variants and 66 heterozygous variants were found after the MT-COX1 gene sequencing of 477 spermatozoa from 2010 to 2021. This includes a newly discovered homozygous variation (m.6534A□G) and 11 heterozygous variants (m.5925T□C, m.5934A□G,

m.5963A□G, m.5967T□C, m.5986T□G, m.6029C□A, m.6250C□A, m.6365T□G, m.7231A□G, m.7245A□C and m.7403A□C). The results of a retrospective study showed that since 2014, the sperm MT-COX1 gene began to show reverse variation at the m.7028C > T locus and 20 site-specific variations (Table 2). With the exception of missense variation of the two sites of m.6366G > A and m.7146A > G in the MT-COX1 gene, the other 18 locus variants were synonymous variations. Among them, the missense variation of m.6366G > A changed proline to isoleucine; the missense variation of m.7146A > G changed threonine to alanine. We predicted the protein function of these two missense variants using the UniProt database and Polyphen-2 software. We found that the probable damaging value of m.6366G > A missense variation was 0.000; the m.7146A > G missense variation had a nominal damaging value of 0.011. Two variants of the probable damaging values of the points were close to 0, which suggests that the variation of these two sites might not have an obvious effect on the protein function. The Swiss-model software was used to model the wild-type and variant MT-COX1 gene-encoded proteins and calculate the RMSD difference between the corresponding high protein structures in the database to evaluate the effect of SNPs on the stability of the protein spatial structure. We found that the wild-type RMSD = 0.1 and the variant RMSD = 1.24, which suggests that the variant protein had lower spatial structure stability than the wild-type protein.

Table 2
21 sperm variation of *MT-COX1* gene in Chinese Han population between 2010–2013 years and 2014–2021 years.

Locus	Nucleotide change	Amino acid change	2010–2013 years (n = 215)		2014–2021 years (n = 262)		P
			N	Frequency (%)	N	Frequency (%)	
6023	G-A	syn	7	3.26%	200	76.33%	0.000[a]
6221	T-C	syn	7	3.26%	203	77.48%	0.000[a]
6242	C-T	syn	4	1.86%	194	74.05%	0.000[a]
6266	A-C	syn	1	0.47%	199	75.95%	0.000[a]
6299	A-G	syn	19	8.84%	204	77.86%	0.000[a]
6366	G-A	V-I	12	5.58%	209	79.77%	0.000[a]
6383	G-A	syn	47	21.86%	192	73.28%	0.000[a]
6410	C-T	syn	9	4.19%	202	77.10%	0.000[a]
6452	C-T	syn	11	5.12%	200	76.33%	0.000[a]
6483	C-T	syn	10	4.65%	199	75.95%	0.000[a]
6512	T-C	syn	12	5.58%	84	72.41%	0.000[b]
6542	C-T	syn	9	4.19%	84	72.41%	0.000[b]
6569	C-A	syn	10	4.65%	84	72.41%	0.000[b]
6641	T-C	syn	13	6.05%	84	72.41%	0.000[b]
6935	C-T	syn	5	2.33%	83	71.55%	0.000[b]
6938	C-T	syn	10	4.65%	83	71.55%	0.000[b]
7028	C-T	syn	213	99.07%	82	70.69%	0.000[b]
7146	A-G	T-A	9	4.19%	83	71.55%	0.000[b]
7232	C-T	syn	21	9.77%	86	74.14%	0.000[b]

a: 2014–2021, n = 262

b: 2014–2018, n = 116

Locus	Nucleotide change	Amino acid change	2010–2013 years (n = 215)		2014–2021 years (n = 262)		P
			N	Frequency (%)	N	Frequency (%)	
7256	C-T	syn	8	3.72%	84	72.41%	0.000[b]
7316	G-A	syn	3	1.40%	84	72.41%	0.000[b]
a: 2014–2021, n = 262							
b: 2014–2018, n = 116							

3.2. MT-COX1 Gene Variation and Time

The MT-COX1 gene containing 20 variation sites (including homozygous variation and heterozygous variation), such as m.6023G > A, was called a genetic variant. The gene containing no variation was called a wild-type gene, i.e., the G/G genotype. Statistical analysis found that the MT-COX1 gene m.6023G > A allele variation rate showed an obvious increasing trend in 2014, so we divided the research subjects into two groups: 2010–2013 and 2014–2021. We found that the variation frequency increased from 3.26% in 2010–2013 to 78.17% in 2014–2021 ($P < 0.05$). Significant reversion variation occurred in the MT-COX1 m.7028C > T locus. The gene variation rate of the m.7028C > T locus in 2010–2013 was significantly higher than that in 2014–2018, i.e., 98.61% vs. 70.87% ($P < 0.05$). In addition, we compared the difference in gene variation among the 27–33 age group between the two groups. The results show that the frequency of gene variation in this stage increased from 2.75–79.79%. In other words, 79.79% of the male spermatozoa between the ages of 27 and 33 have undergone genetic variation.

3.3. Inherited and Acquired Variations

To further clarify that the variant MT-COX1 gene was not derived from the mother's inheritance but acquired variations, we collected the sperm and peripheral venous blood samples of six married fertile male volunteers and peripheral blood of their own mothers. The MT-COX1 sequencing results show that 5 of the 6 spermatozoa had MT-COX1 gene variation, and only 1 was still the wild-type MT-COX1 gene. However, the MT-COX1 genes in the peripheral blood of 6 male peripheral venous blood samples and their mothers were all wild-type, and no genetic variation occurred (Fig. 1).

3.4. MT-COX1 Gene Variation and Age

To assess whether age contributes to MT-COX1 gene variations in male spermatogenesis, we compared the age and gene (m.6023G > A and m.7028C > T) variants between the 2010–2013 group and the 2014–2018 group by logistic regression analysis. The correlation logistic regression analysis indicates that age was not a relevant factor that caused the sperm mitochondrial gene variation (data not shown).

3.5. Variation Rate and Time of the MT-COX1 Gene

All samples were classified according to the time of variation, and the variation rate was calculated (Supplemental Fig. 1). Excluding several years with a small sample size, we can determine that the variation rate of sperm MT-COX1 has sharply increased since 2014. We compared the variation rate between 2010–2013 and 2014–2021. Among 477 samples, the variation group accounted for 7 cases (3.26%) in 2010–2013 and 210 cases (80.15%) in 2014–2021. Chi-square test results show that the variation rate in 2014–2021 was higher than that in 2010–2013, and the difference was statistically significant ($P < 0.05$) (Fig. 2A).

3.6. MT-COX1 Gene and Sperm Quality

To study the effect of the MT-COX1 gene variation at m.6023G > A on the sperm quality, we prospectively analyzed 146 sperm samples of individuals aged 27–33 years old from 2019 to 2021. According to the sequencing results, the sperm samples were divided into three groups: G/G genotype (wild type, n = 18), G/A genotype (n = 77) and A/A genotype (n = 51). The constituent ratios were 12.33%, 52.74% and 34.93%, respectively. A prospective study shows that there was no significant difference in age between (G/G + G/A) genotypes and A/A genotype (30.00 (28.00–31.00) years old for (G/G + G/A) versus 30.00 (29.00–31.50) years old for A/A; P = 0.96). There were significant differences in sperm density and motility between (G/G + G/A) genotypes and A/A genotype. The A/A genotype had higher sperm density than the G allele carrier (genotype A/A: sperm density (10^6/ml) = 58.53 ± 2.85 versus genotype (G/G + G/A): sperm density (10^6/ml) = 50.76 ± 2.54; P = 0.041). The A/A genotype had higher sperm motility than the G allele carrier (genotype A/A: sperm motility (%) = 46.72 (43.32–54.07) versus genotype (G/G + G/A): sperm motility (%) = 44.05 (31.45–52.00);P = 0.037) (Figs. 2B, 2C and 2D).

According to the WHO laboratory manual for the examination and processing of human semen (2010), we divided the sperm samples into two groups: oligospermia / asthenospermia group (density < 15 × 10^6/ml or motility < 32%) and normal group. We find that the incidence of oligospermia / asthenospermia in G allele carrier type (G/G + G/A) was higher than that in A/A genotype (Table 3).

Table 3
Frequency of oligospermia / asthenospermia between the two groups

	A/A genotype	G allele carrier type(G/G + G/A)	Frequency	P
total amount	51	95		
oligospermia / asthenospermia	6	26	11.76%[a] 27.37%[b]	0.030
a: Frequency of oligospermia / asthenospermia in A/A genotype;				
b: Frequency of oligospermia / asthenospermia in G allele carrier type (G/G + G/A).				

3.7. Oligoasthenoteratospermia and Genomics

We selected 13 normal sperm samples and 4 oligoasthenoteratospermia sperm samples for low-throughput sequencing of the whole sperm genome. We found that the DNAAF6 gene (also known as PIHID3) had different SNP sites between the two groups. We found that oligoasthenoteratospermia group contains 14 unique SNP loci, of which 5 were newly discovered SNP loci (Supplemental Table 1), except for 107243978 in exon of DNAAF6 gene, all the other SNP loci were in intron. Through sequence alignment at site 107243978, we found that it was located in the 3'UTR sequence region of the transcript of the DNAAF6 gene. Through prediction, we found that this site did not bind to miRNA, but hsa-miR-4311 might bind to the 3'UTR in the upstream of this site.

4. Discussion

From the results of 477 valid sperm samples of the MT-COX1 gene, we first found that the MT-COX1 gene of spermatozoa of the Chinese Han population has varied since 2014. m.7028C > T is a ubiquitous haplotype marker in human populations, and only the European H group did not have this site variation. Analysis of 20 MT-COX1 gene variations revealed that there were missense variations in the two sites of m.6366G > A and m.7146A > G. The amino acid changes of the gene might cause changes in the spatial structure of the protein, which affects the function of the protein. Therefore, we performed three-dimensional modeling of proteins using the Swiss-model, Uniport database and Polyphen-2 to predict the function of proteins and calculate the structural stability of wild-type and variant gene-encoded proteins. The prediction results suggest that although the amino acids corresponding to the two sites of m.6366G > A and m.7146A > G changed, the amino acids of the variation were still located in the helical structure, so the effect on the overall structure of the protein was not obvious.

We found that the reverse variation at the m.7028C > T locus and 20 site-specific variations began to appear in 2014 and showed a significant increasing trend. Because the mitochondrial gene was still considered matrilineal inheritance, we first recruited six male volunteers to test the semen and blood samples from them and blood samples from their biological mother. The results show that this group of gene variations only occurred in sperm mitochondria, i.e., the variation in sperm mitochondria is caused by some acquired factor.

The spermatozoa of 5 male volunteers in 2012 and 2018 were analyzed to understand the changes in sperm mitochondrial genes at an interval of 5–6 years. The entire genome of nuclear genes in 2 of the 5 samples that satisfy the requirements of detection was sequenced by whole genome sequencing. Mitochondrial DNA polymerase γ encoded by the human nuclear gene is the only known enzyme that can repair mitochondrial DNA damage. The results show that the single nucleotide polymorphism and deletion of the human sperm mt-DNA polymerase γ gene were not related to the variation in the mt-DNA gene (data not shown). However, the decrease in mitochondrial Poly-γ polymerase activity caused by the post-transcriptional modification of the POLG gene should not be ruled out. Later, we will explore whether modifications such as POLG gene methylation can affect the activity of mitochondrial Poly- γ polymerase.

Lehtinen et al. found that certain pathogenic mt-DNA variants have directional drift (increased or decreased) or are maintained at a stable heterogeneity level in different cell lines [14]. Krjutškov et al. found that the m.16093A > C variation in the testis was up to 90% and only approximately 10% in skeletal muscle [15]. In 2003, the site of genetic variation in sperm mitochondria still appeared wild in the blood [16]. The study found that the MT-COX1 gene had eight gene variation sites, such as m.6241C > T, but these eight variation sites did not appear in our study. Li et al. found that with age, specific genetic variations in specific tissues of individuals were positively screened and finally formed specific genotypes [17].

We divided all samples into two groups according to age ≤ 30 years old and age > 30 years old for the differential analysis. The results show that the genetic variation and age were not correlated ($P > 0.05$). In addition, we statistically analyzed the sperm variation rate from 2010 to 2021 and found that the sperm variation rate sharply increased from 2014. Therefore, the difference analysis was performed in the 2010–2013 group and 2014–2021 group. The variation rate was higher in 2014–2021 than that in 2010–2013, and the difference was statistically significant. In other words, the proportion of variant MT-COX1 genes in the population has sharply increased in recent years.

In our prospective study, we found that the sperm quality, sperm density and motility of the MT-COX1 A/A genotype were higher than those of the G allele carrier (G/A + G/G). A previous study has found that the severity of pathogenic variation varies among individuals, i.e., there is variable penetrance [18]. Regarding the penetrance rate, the gene variation that controls gene activity may modify the disease risk caused by the variation of protein-coding genes. When studying the relationship between mitochondrial ND3, ND4L nucleotide variation and asthenospermia, Li et al. found that the variation rate of missense variation m.10398A > G in the normal control group was higher than that in the asthenospermia group [19]. Regarding the m.10398A > G variation, the amino acid changed from threonine to alanine, which was consistent with our m.7146A > G amino acid change. They speculated that this variation changed the enzyme activity and might be beneficial to energy metabolism.

DNAAF6 (Dynamic assembly factor 6) mainly provides the regulation of protein complex for cilia dynamic protein. We found that the DNAAF6 gene on X chromosome had 14 unique SNP sites in oligoasthenoteratospermia group, of which site 107243978 was located in the exon sequence of the gene, which was the 3'UTR region of DNAAF6 gene transcript. At present, our main understanding of the function of 3'UTR was as follows: 3'UTR could regulate the localized expression of mRNA, regulate the translation of mRNA, bind to multiple RNA binding proteins to regulate function, and regulate protein-protein interaction. Therefore, we believed that the variation of this site might lead to mislocalization and expression of DNAAF6 transcripts, resulting in abnormal degradation of transcripts and insufficient expression of DNAAF6 protein or translation obstacles. At the same time, the variation of intron might also affect the stability and spatial structure of mature mRNA by affecting the splicing of precursor mRNA.

In 1992, Carlsen et al. statistically analyzed the human sperm quality during the 50 years from 1940 to 1990 and found that the sperm density and semen volume were significantly reduced [1]. We collected 18 pieces of literature on the semen quality since 1938[1, 20–36], collected data on the sperm quality worldwide for analysis, and found that the sperm density and motility had indeed shown a downward trend in recent years (Supplemental Fig. 2).

Through the above analysis, we can still think that the decline of human sperm quality has existed for a long time. The MT-COX1 gene of spermatozoa of the Chinese Han population had varied at 20 specific sites since 2014. Among them, the sperm quality of the A/A genotype at the m.6023G > A locus was better than that of G allele carriers (G/G + G/A). At the same time, the variation at position 107243978 of DNAAF6 gene might be a risk factor for oligoasthenoteratospermia.

Declarations

1. **Funding:** This study was supported by Health Commission of Henan Province (Grant numbers [2018010009]).
2. **Competing Interests:** The authors have no relevant financial or non-financial interests to disclose.
3. **Author Contributions:** All authors contributed to the study conception and design. Xiaoxiao Li and Hongyang Chang are responsible for experimental design, experimental research, writing the draft and data analysis. Bo Cai is responsible for data analysis. Linghan Zhang, Le Zhang and Peipei Guo are responsible for collect raw data. Bing Xiao, Qian Li, Jing Liu and Lili Tong are responsible for participate in experimental design. Xiaoting Xu is responsible for data management. Genhong Mao is responsible for guide the design of the experiment, revise and polish the paper. All authors read and approved the final manuscript
4. **Ethics approval:** This research project was approved by the Ethics Committee of the Second Affiliated Hospital of Zhengzhou University (Ethics batch number: 2021154).
5. **Consent to participate:** Signed and Informed consent was obtained from all individual participants included in the study.
6. **Consent to publish:** The authors affirm that research participants provided informed consent for publication.

References

1. Carlsen E, Giwercman A, Keiding N et al (1992) Evidence for decreasing quality of semen during past 50 years. BMJ 305(6854):609–613. doi:10.1136/bmj.305.6854.609
2. Albers P, Albrecht W, Algaba F et al (2012) Guía clínica sobre el cáncer de testículo de la EAU: actualización de 2011 [EAU guidelines on testicular cancer: 2011 update. European Association of Urology]. Actas Urol Esp 36(3):127–145. doi:10.1016/j.acuro.2011.06.017

3. Levine H, Jørgensen N, Martino-Andrade A et al (2017) Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update 23(6):646–659. doi:10.1093/humupd/dmx022
4. Talebi E, Karimian M, Nikzad H (2018) Association of sperm mitochondrial DNA deletions with male infertility in an Iranian population. Mitochondrial DNA A DNA Mapp Seq Anal 29(4):615–623. doi:10.1080/24701394.2017.1331347
5. Gholinezhad Chari M, Hosseinzadeh Colagar A, Bidmeshkipour A (2015) A novel large-scale deletion of the mitochondrial DNA of spermatozoa of men in north iran. Int J Fertil Steril 8(4):453–463. doi:10.22074/ijfs.2015.4185
6. Bahrehmand Namaghi I, Vaziri H (2017) Sperm mitochondrial DNA deletion in Iranian infertiles with asthenozoospermia. Andrologia 49(3). 10.1111/and.12627.. doi:10.1111/and.12627
7. Ambulkar PS, Chuadhari AR, Pal AK (2016) Association of large scale 4977-bp "common" deletions in sperm mitochondrial DNA with asthenozoospermia and oligoasthenoteratozoospermia. J Hum Reprod Sci 9(1):35–40. doi:10.4103/0974-1208.178635
8. Ambulkar PS, Waghmare JE, Chaudhari AR et al (2016) Large Scale 7436-bp Deletions in Human Sperm Mitochondrial DNA with Spermatozoa Dysfunction and Male Infertility. J Clin Diagn Res 10(11):GC09–GC12. doi:10.7860/JCDR/2016/22412.8843
9. Mughal IA, Irfan A, Hameed A et al (2016) Sperm mitochondrial DNA 15bp deletion of cytochrome c oxidase subunit III is significantly associated with human male infertility in Pakistan. J Pak Med Assoc 66(1):3–7
10. Mughal IA, Irfan A, Jahan S et al (2017) Male infertility is significantly associated with multiple deletions in an 8.7-kb segment of sperm mtDNA in Pakistan. Turk J Med Sci 47(3):928–933 Published 2017 Jun 12. doi:10.3906/sag-1606-52
11. Mao GH, Wang YN, Xu M et al (2015) Polymorphisms in the MT-ATP6 and MT-CYB genes in in vitro fertilization failure. Mitochondrial DNA 26(1):20–24. doi:10.3109/19401736.2013.840612
12. Mao G, Lu P, Huang XH et al (2016) The analysis of mitochondrial DNA haplogroups and variants for in vitro fertilization failure in a Han Chinese population. Mitochondrial DNA A DNA Mapp Seq Anal 27(4):2993–3000. doi:10.3109/19401736.2015.1060476
13. Mao GH, Huang XH, Geng XJ et al (2020) Correlation between sperm mitochondrial ND5 and ND6 gene variations and total fertilisation failure. Arch Med Sci 16(3):692–698 Published 2020 Apr 25. doi:10.5114/aoms.2020.94658
14. Lehtinen SK, Hance N, El Meziane A et al (2000) Genotypic stability, segregation and selection in heteroplasmic human cell lines containing np 3243 mutant mtDNA. Genetics 154(1):363–380. doi:10.1093/genetics/154.1.363
15. Krjutškov K, Koltšina M, Grand K et al (2014) Tissue-specific mitochondrial heteroplasmy at position 16,093 within the same individual. Curr Genet 60(1):11–16. doi:10.1007/s00294-013-0398-6
16. Thangaraj K, Joshi MB, Reddy AG et al (2003) Sperm mitochondrial mutations as a cause of low sperm motility. J Androl 24(3):388–392. doi:10.1002/j.1939-4640.2003.tb02687.x

17. Li M, Schröder R, Ni S et al (2015) Extensive tissue-related and allele-related mtDNA heteroplasmy suggests positive selection for somatic mutations. Proc Natl Acad Sci U S A 112(8):2491–2496. doi:10.1073/pnas.1419651112
18. Castel SE, Cervera A, Mohammadi P et al (2018) Modified penetrance of coding variants by cis-regulatory variation contributes to disease risk. Nat Genet 50(9):1327–1334. doi:10.1038/s41588-018-0192-y
19. Chuanlian L, Zhefeng L, Xuefeng H et al (2010) Association between asthenospermia and mtDNA mutations in ND3 and ND4L genes. Chin J Pathophysiology 26(2):362–367 Chinese
20. Irvine S, Cawood E, Richardson D et al (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. BMJ 312(7029):467–471. doi:10.1136/bmj.312.7029.467
21. Srám RJ, Benes I, Binková B et al (1996) Teplice program–the impact of air pollution on human health. Environ Health Perspect 104(Suppl 4):699–714. doi:10.1289/ehp.104-1469669
22. Swan SH, Brazil C, Drobnis EZ et al (2003) Geographic differences in semen quality of fertile U.S. males. Environ Health Perspect 111(4):414–420. doi:10.1289/ehp.5927
23. Dissanayake D, Wijesinghe P, Ratnasooriya W et al (2010) Relationship between seminal plasma zinc and semen quality in a subfertile population. J Hum Reprod Sci 3(3):124–128. doi:10.4103/0974-1208.74153
24. Mendiola J, Jørgensen N, Andersson AM et al (2011) Jan;119(1):A11] Are environmental levels of bisphenol a associated with reproductive function in fertile men? [published correction appears in Environ Health Perspect. Environ Health Perspect. 2010;118(9):1286–1291. doi:10.1289/ehp.1002037
25. Fernandez MF, Duran I, Olea N et al (2012) Semen quality and reproductive hormone levels in men from Southern Spain. Int J Androl 35(1):1–10. doi:10.1111/j.1365-2605.2010.01131.x
26. Rao M, Meng TQ, Hu SH et al (2015) Evaluation of semen quality in 1808 university students, from Wuhan, Central China. Asian J Androl 17(1):111–116. doi:10.4103/1008-682X.135984
27. Halling J, Petersen MS, Jørgensen N et al (2013) Semen quality and reproductive hormones in Faroese men: a cross-sectional population-based study of 481 men. BMJ Open. ;3(3):e001946. Published 2013 Mar 1. doi:10.1136/bmjopen-2012-001946
28. Mínguez-Alarcón L, Chavarro JE, Mendiola J et al (2014) Physical activity is not related to semen quality in young healthy men. Fertil Steril 102(4):1103–1109. doi:10.1016/j.fertnstert.2014.06.032
29. Lao XQ, Zhang Z, Lau AKH et al (2018) Exposure to ambient fine particulate matter and semen quality in Taiwan. Occup Environ Med 75(2):148–154. doi:10.1136/oemed-2017-104529
30. Liu Y, Zhou Y, Ma J et al (2017) Inverse Association between Ambient Sulfur Dioxide Exposure and Semen Quality in Wuhan, China. Environ Sci Technol 51(21):12806–12814. doi:10.1021/acs.est.7b03289
31. Mishra P, Negi MPS, Srivastava M et al (2018) Decline in seminal quality in Indian men over the last 37 years. Reprod Biol Endocrinol. ;16(1):103. Published 2018 Oct 23. doi:10.1186/s12958-018-0425-

z

32. Khayamabed R, Tavalaee M, Taherian SS et al (2020) Effect of recombinant β-defensin 1 protein on human sperm motility and viability. Andrologia 52(1):e13455. doi:10.1111/and.13455
33. Moscatelli N, Lunetti P, Braccia C et al (2019) Comparative Proteomic Analysis of Proteins Involved in Bioenergetics Pathways Associated with Human Sperm Motility. Int J Mol Sci 20(12):3000 Published 2019 Jun 19. doi:10.3390/ijms20123000
34. Ghaffarilaleh V, Fisher D, Henkel R (2019) Carica papaya seed extract slows human sperm. J Ethnopharmacol 241:111972. doi:10.1016/j.jep.2019.111972
35. Aram R, Chan PTK, Cyr DG (2020) Beta-defensin126 is correlated with sperm motility in fertile and infertile men†. Biol Reprod 102(1):92–101. doi:10.1093/biolre/ioz171
36. Banihani SA, Shatnawi RM (2020) Aspirin decreases human sperm motility and vitality, chelates seminal calcium, but insignificantly reduces seminal nitric oxide production. Andrologia 52(10):e13776. doi:10.1111/and.13776

Figures

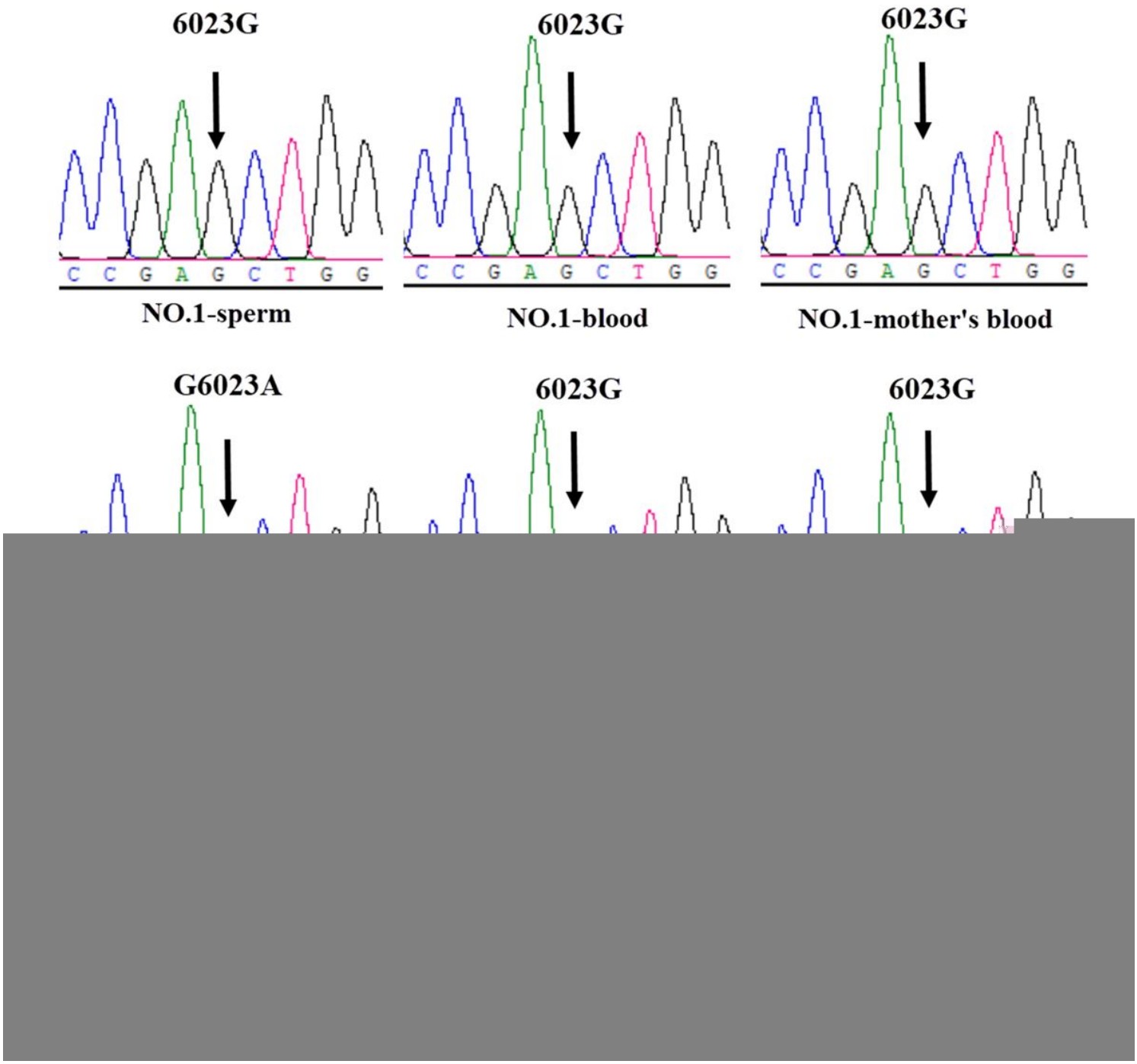

Figure 1

m.6023G waveforms of male spermatozoa, venous blood and mother's venous blood in the three groups. All m.6023G in male spermatozoa, venous blood and maternal venous blood of No. 1 were wild-type (G/G); G/A genotype occurred at m.6023G of spermatozoa in men No. 4 and No. 6, and wild-type (G/G) was found in venous blood and mother's venous blood.

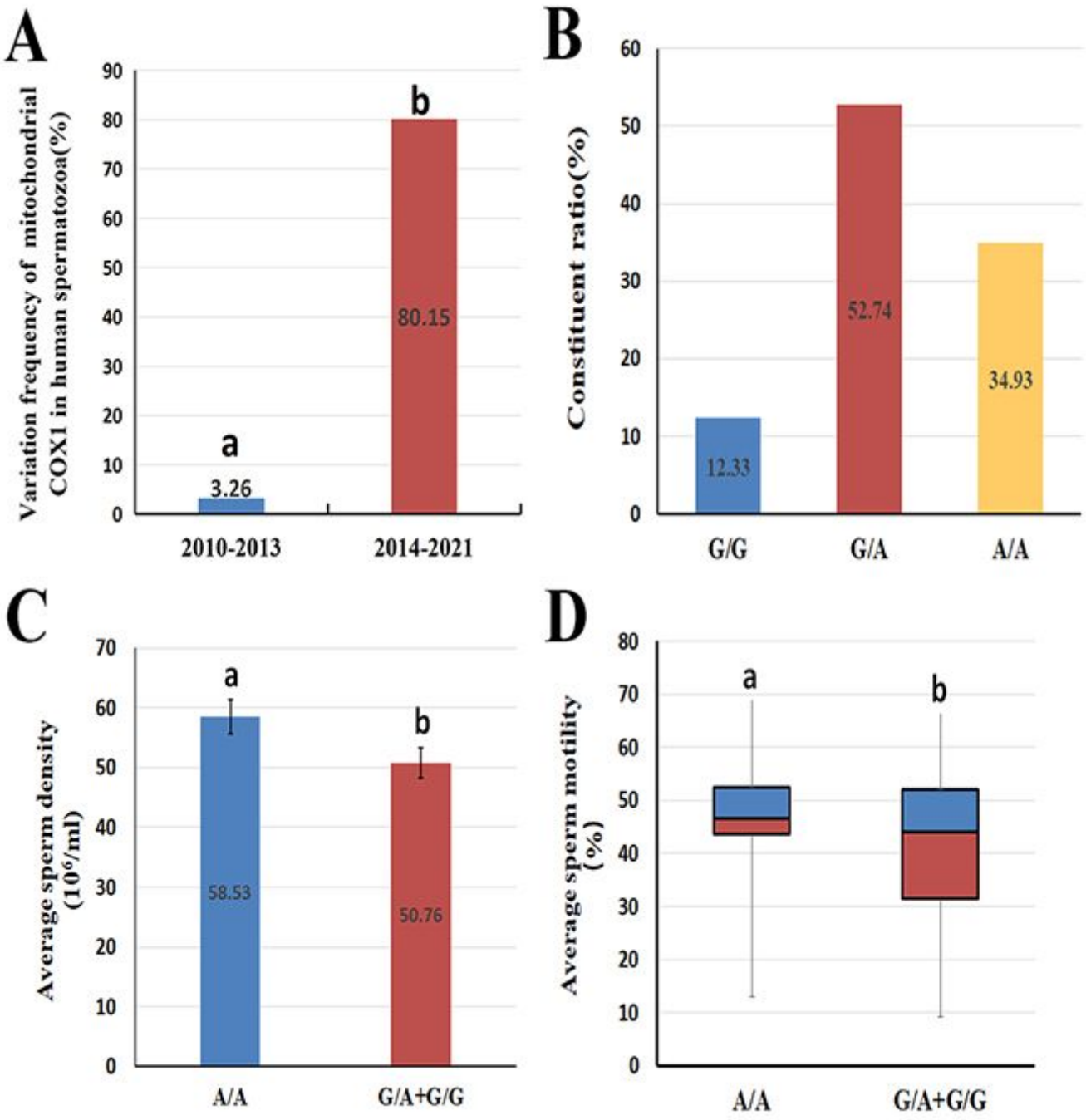

Figure 2

Variation of *MT-COX1* in spermatozoa. (A) Sperm MT-COX1 variation frequency between the 2010-2013 and 2014-2021. (B) Sperm mitochondrial variation constituent ratio during 2019-2021. (C) Average sperm density during 2019-2021. (D) Average sperm motility during 2019-2021. Letters a and b represent the two groups with statistically significant differences.

Supplementary Files

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